Direct Molding of Plastics from
Casein and Carbamido Casein*

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Molding powders prepared from casein and carbamido casein, which are prehardened with formaldehyde, can be readily molded into simple objects having good strength. With the aid of an organic plasticizer and with a maximum of 10% water in the molding powder, these materials can be molded into specimens which have dimensional stability under ordinary atmospheric conditions. Chemical modification of casein to carbamido casein materially improves the water absorption of the protein, yet does not destroy its adhesive properties. The possible value of carbamido casein for use in fields other than plastics has not been fully explored. A new tensile strength test specimen with appropriate specimen holders is also described.
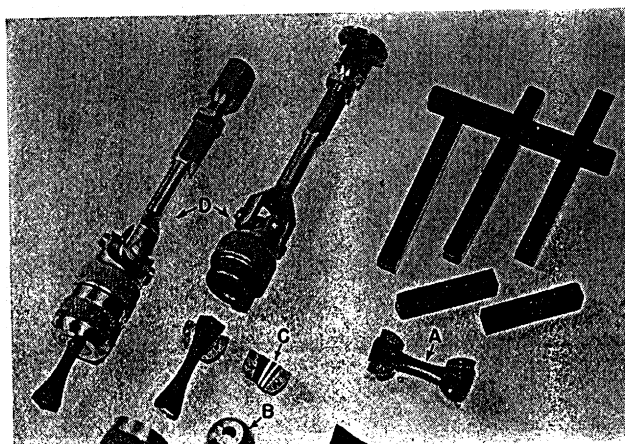
CASEIN plastics have maintained a good, though limited, position for many years, owing principally to the range, depth, and stability of their color. The process of manufacture in general use[1] has been little affected by the rapid strides made in the molding of other types of plastics. The maker of casein plastics would like to increase his rate of turnover by eliminating, for example, the several days used for curing the product in a formaldehyde bath. Since direct molding would offer a solution for some of the difficulties in the present manufacturing scheme, plasticizers and synthetic resins as well as chemical modification have been employed in numerous attempts to prepare satisfactory molding powders from proteins. As compared with the shaping machines in use at present, which can be adjusted to turn out many different types of protein plastic buttons, molds for this purpose would not be an unmixed blessing. They would need to be multi-cavity and therefore would be expensive and would require heavy and prolonged production to cover their cost. Nevertheless, the advantages of direct molding appear sufficient to justify continued efforts to find successful means of accomplishment.

If hardening of protein plastics is to be avoided following molding, it must be performed in some manner before or during molding. Substances which might give delayed hardening have received considerable attention, but we wish here to deal with materials hardened before molding. Formaldehyde has been used, for example,[2, 3, 4, 5] to preharden

[2]German Patent 523,947, April 30, 1931, by Heinz Busse.
[3]"Indurated albuminoid compound," by B. B. Goldsmith, U. S. Patent 1,027,121, May 21, 1912. "Indurated casein compounds," by B. B. Goldsmith, U. S. Patent 1,027,122, May 21, 1912.
[4]"Manufacture of articles for proteins," by H. Plauson, assignor to D. R. Rotman, U. S. Patent 1,395,729, Nov. 1, 1921.
[5]German Patent 489,438, Jan. 17, 1930, by P. Stock.

1 — Tensile strength test specimens and specimen holder.
A: Tensile strength specimen, 0.200-in. diameter at minimum point with 3-in. radius of curvature. B: Specimen holder, ball-swivel joint insert. C: Ball-swivel joint insert, surface corresponding to the curvature of specimen. D: Specimen holder, universal joints

Specimen	Material[b]	Testing machine used		Differences by reference to Tinius Olsen values for 0.200 in. spindles			
		Modified Scott	Tinius Olsen	Modified Scott		Tinius Olsen	
		p.s.i.	p.s.i.	p.s.i.	%	p.s.i.	%
Spindle 0.160-in. diam.	Casein		8990			+ 90	+ 1.0
	Carbamido casein		8940			None	0.0
	Oleoyl casein		5330			— 80	— 1.5
Spindle 0.200-in. diam.	Casein	9240	8900[c]	+340	+3.8		
	Carbamido casein	9380	8940[c]	+440	+4.9		
	Oleoyl casein	5710	5410[c]	+300	+5.5		
Spindle 0.312(5/16)-in. diam.	Casein		8430			— 470	— 5.3
	Carbamido casein		8560			— 380	— 4.3
	Oleoyl casein		4650			— 760	—14.0
Dumbbell 0.125(1/8) in. by 1 in. wide	Casein		9240			+ 340	+ 3.8
	Carbamido casein		7970			— 970	—10.9
	Oleoyl casein		4310			—1100	—20.3
Dumbbell 0.200 in. thick by 1 in. wide	Casein		8500			— 400	— 4.4
	Carbamido casein		7720			—1220	—13.6
	Oleoyl casein		3760			—1650	—30.5

[a]The values given are the average of six to eight results.
[b]All the materials were hardened with formaldehyde before molding. The casein and carbamido casein (100 parts casein treated with 10 parts of KCNO) were hardened in solution with 4% formaldehyde. The oleoyl casein has been described by Gordon et al. (reference 27, sample 38-39 in Table V).
[c]These are the values used for reference.

casein for use in molding powders; Brother and McKinney[6], [7] have reported extensive experiments of this type, particularly with soybean protein. The effect of formaldehyde, however, is to increase the

[6]"Protein plastics from soybean products. Action of hardening or tanning agents on protein material," by G. H. Brother and L. L. McKinney, Ind. Eng. Chem. 30, 1236-1240 (1938).
[7]"Process for producing protein plastics," by G. H. Brother and L. L. McKinney, assignors to Secretary of U. S. Dept. of Agric., U. S. Patent 2,309,380, Jan. 26, 1943.

Table II.—Variations of Tensile Strength with Water Content of Carbamido Casein[a]

Water content at molding	Conditioning treatment	Approx. water content in spindle portion at time of test[b]	Tensile strength[c]
%		%	p.s.i.
20	4 wk., in oven at 50° C.	6.3	10,600
20	1 wk., in oven at 50° C.	8.7	9,700
20	1 wk., 31% R.H., 25° C.	9.9	9,450
20	4 wk., 31% R.H., 25° C.	8.2	9,300
20	1 wk., 50% R.H., 25° C.[d]	10.9	9,050
20	4 wk., 50% R.H., 25° C.[d]	11.0	9,000
20	1 wk., 81% R.H., 25° C.	13.9	6,950
20	4 wk., 81% R.H., 25° C.	14.2	6,850
14.4	None	14.4[e]	6,550
19.2	None	19.2[e]	4,100
25.0	None	25.0[e]	2,050

[a]The carbamido casein was made by treating 100 parts casein with 10 parts KCNO, and the product was hardened in solution with 4% formaldehyde.
[b]These values (except the last three) are the average values of three spindles from three different bars, obtained by heating to constant weight at 105° C. Samples were taken from the broken portions of the spindles immediately after testing.
[c]Tensile strength values are the average results of six tests on three separate molded specimens. The specimens were tested at 65 ± 2% R.H. at 21 ± 1° C. immediately after removal from the preconditioning atmosphere.
[d]Note A.S.T.M. conditions of 50% R.H. at 25° C. (reference 13).
[e]Actual water contents were not redetermined for these specimens, although a small amount of water was undoubtedly lost in their preparation.

viscosity of wet protein material, and there is, in fact, a definite limit on the amount of formaldehyde compatible with extrusion or molding under reasonable conditions of temperature, pressure and moisture content. For this reason, we have included in this investigation a number of experiments on a modified casein, namely, carbamido casein. This may be regarded as essentially having the basic amino groups replaced with the weakly basic urea groups. Certain properties of carbamido casein are improved by formaldehyde, which it readily takes up, and flow, although lowered, is not affected to the same degree as in the original casein treated with formaldehyde. Another reason for selecting carbamido casein for study was the hope that the urea group might lend itself for condensation with formaldehyde in the same manner as in the polymerization of urea formaldehyde resin.

Testing procedures

Water absorption—Two methods were used. For basic comparison of the water uptake of plastic disks molded from the various protein materials, the water content at saturation (25 ±0.5° C.) was determined according to Mellon[8]. The American Society for Testing Materials procedure[9] was also applied to give a more common characterization.

Tensile strength—Owing to the lack of a standard testing machine for plastics, a Scott J-2 yarn tester

[8]"Determination of the saturation water content of protein plastics," by E. F. Mellon, Ind. Eng. Chem., Anal. Ed. 17, 743 (1945).
[9]"Standard method of test for water absorption of plastics," A.S.T.M. Designation: D 570-42.

Table III.—Effect of Pretreatment of Casein on Formaldehyde-Hardening and on the Properties of the Molded Product

Exp. No.	Hardening of casein with formaldehyde[a]		A.S.T.M. 24-hr. water absorption	Approx. satn. water content	Tensile strength
	Treatment of casein	Condition of casein just before addition of CH₂O			
			%	%	p.s.i.
C-32	None	Commercially dried	11.1	30.8	7,220
C-73	2 hr. at 85° C. and pH 8.7; then pptd. and dried at 40° C.	Dried	13.9	32.2	8,450
C-31	2 hr. at 85° C. and pH 8.7; then pptd. and held moist	Swollen	11.4	30.9	9,540
C-74	3 hr. in water at room temp.	Partially swollen	14.3	32.6	8,600

[a]Hardened in suspension with 10% formaldehyde for three days at room temperature at final pH 4.4.
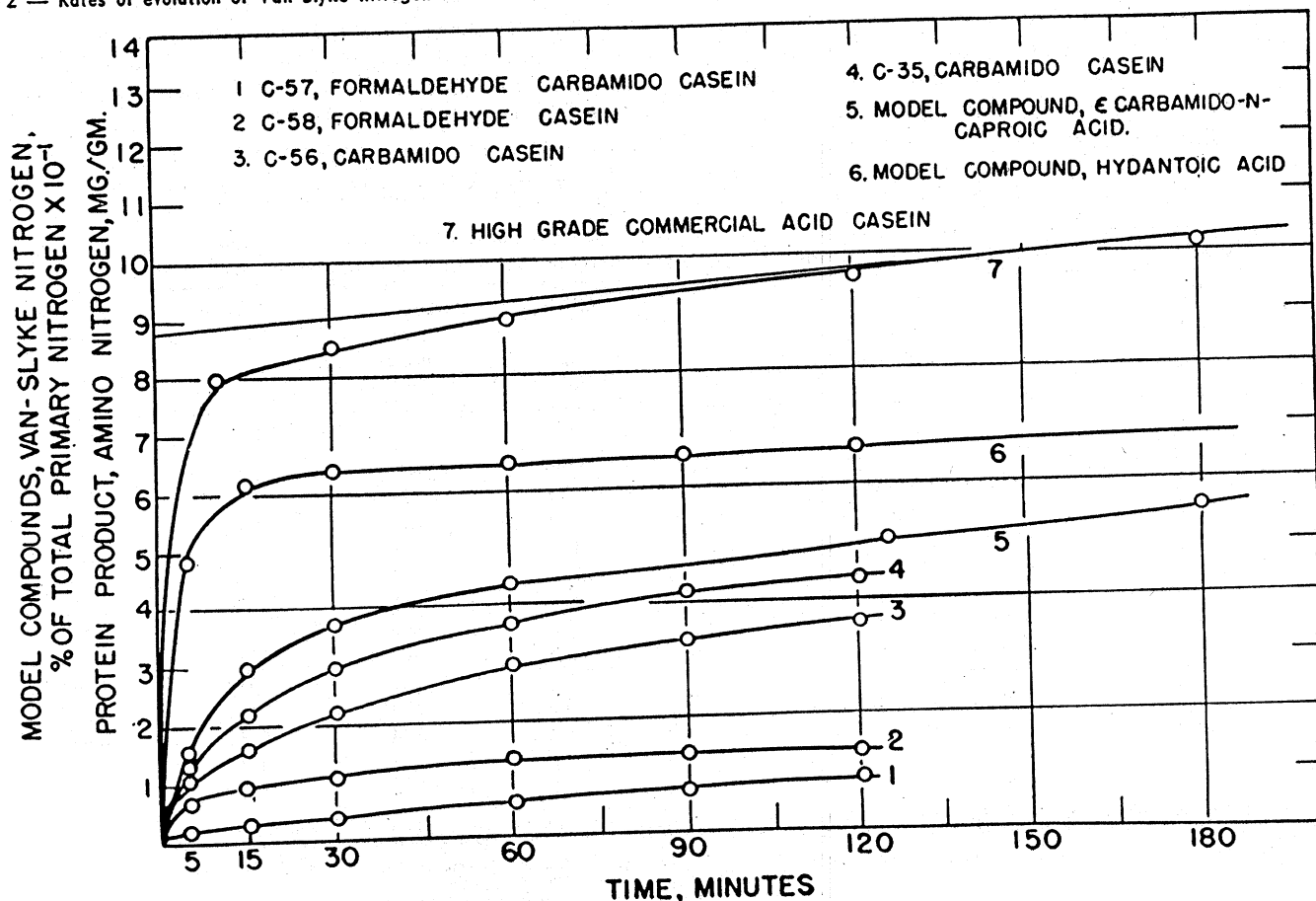
was modified to meet A.S.T.M.[10] requirements for measuring the tensile strength of the plastic specimens. The screw speed of the tester was changed from 12.9 in. per min. to 0.25 in. per min. by means of sprockets and a motor with geared head. Also, the capacity of the testing machine was increased to 440 lb., and calibration was done by the dead weight method. The rate of loading with the machine running idle was 75 lb. per minute. The actual mean rate of loading of a casein plastic specimen with a diameter of 0.200 in. was 2440 p.s.i. per minute. Tensile strength test specimens (A.S.T.M.[11])

[10]"Tentative method of test for tensile properties of plastics." A.S.T.M. Designation: D 638-44T.

[11]"Tentative method of test for flexural strength of electrical insulating materials." A.S.T.M. Designation: D 650-42T.

2 — Rates of evolution of Van-Slyke nitrogen from casein and carbamido derivatives

1 C-57, FORMALDEHYDE CARBAMIDO CASEIN
2 C-58, FORMALDEHYDE CASEIN
3. C-56, CARBAMIDO CASEIN
4. C-35, CARBAMIDO CASEIN
5. MODEL COMPOUND, ∈ CARBAMIDO-N-CAPROIC ACID.
6. MODEL COMPOUND, HYDANTOIC ACID
7. HIGH GRADE COMMERCIAL ACID CASEIN

were obtained from flexural strength test bars. The bar was cut in half, and each half (0.5 by 0.5 by 2.5 in.) was turned down to form the final specimen (Fig. 1). For machining the specimen in the lathe, a sharp cutting tool was pivoted on a 3-in. radius, and the spindle specimen was routinely made with a diameter of 0.200 ±0.004 in. at the minimum point. The specimen may be turned to any reasonable thickness as long as the load required to break the specimen is within the capacity of the testing machine. Specimens with diameters as low as 0.160 in. have given excellent check values, and with few exceptions have fractured at the minimum diameter. Self-aligning grips were especially designed to hold the specimen. The ball (Fig. 1) of the ball-swivel joint of the grip that actually bears on the shoulder of the specimen is split, and its inner curvature is made to conform as closely as possible to the corresponding curvature of the specimen. Obviously, the fit of the test piece in a given ball insert depends on the diameter of the specimen, and a bad fit might induce stresses leading to unsatisfactory fractures at break. Only two units, constructed with exact fit for specimens of 0.200 and 0.250-in. minimum diameters, respectively, were required. These provided sufficient range of bearing surface to give satisfactory tests with specimens of 0.160 to 0.320-in. minimum diameter. In addition to the ball-swivel joints, the specimen holder was constructed with two additional universal joints (Fig. 1) insuring that self-aligning would occur and that the specimens would not be subject to shear stresses.

It has been possible recently to compare the tensile strength values obtained with the modified Scott J-2 yarn tester on machined spindle specimens with those obtained with a universal testing machine (Tinius Olsen "Plastiversal") both on standard A.S.T.M.[12] dumbbell specimens and on machined spindle specimens (Table I). After conditioning, all the specimens were tested at the same mean rate of loading, that is, 2440 p.s.i. per min., which was the mean rate of loading originally employed with 0.200-in. diameter spindles on the Scott machine. To maintain this constant mean rate of loading, the crosshead speed of the universal testing machine was varied from 0.02 in. per min. for 0.312-in. diameter spindle to 0.07 in. per min. for the 0.200-in. thick dumbbells.

Table I shows good agreement between 1) values for spindles tested in the two different machines, 2) values for spindles of different diameters, especially for 0.160 and 0.200-in. diameters, and 3) values for spindles and dumbbells made from formaldehyde-hardened casein. The carbamido casein and oleoyl casein molded into dumbbell specimens gave markedly lower values than the same material in the form of spindles. In general, the spread for the individual values of a series was less for spindle specimens than for dumbbell specimens. Furthermore, the results obtained with the modified Scott machine on 0.200-in. spindles proved reliable.

The effect of water content on the physical properties of protein materials is important, and the data presented in Table II show that the tensile strength of carbamido casein ranges from 10,600 p.s.i. for a 6.3% water content down to 2050 p.s.i. for 25% water. When the results are plotted, they reveal a fairly close inverse relationship over the range studied. At the higher concentrations of water, 19.2 and 25%, the breaking elongation is pronounced, and the specimen is soft and flexible. These findings are qualitatively in line with the well-known reduction of tensile strength by plasticizers in general.

The extremely great dependence of strength on

[12] "Tentative method of test for tensile strength of molded electrical insulating materials." A.S.T.M. Designation: D 651-42T.

Table IV.—Physical Properties of Plastics Molded from Formaldehyde-Hardened Casein and Carbamido Casein

Exp. No.	KCNO per 100 g. casein	Hardened with CH_2O[a]	A.S.T.M. 24-hr. water absorption	Approx. satn. water content	Tensile strength	Flexural strength
	g.		%	%	p.s.i.	p.s.i.
C-96	0	None[b]	31.7	42.9	....	....
C-30	0	Solution, 1-step[b]	15.3	33.8	9250	8,600
C-45	0	Solution, 2-step[b]	17.8	33.8	9260	9,740
C-53	0	Solution, 1-step and suspension[a]	17.9	32.6	9840	9,200
C-13	5	Solution, 2-step	11.7	29.7	9550	10,500
C-43	5	Solution, 2-step	10.2	28.9	9200	9,490
C-10	10	Solution, 2-step	7.2	25.9	9350	7,600
C-42	10	Solution, 2-step	7.8	26.6	9210	9,490
C-20	10	Solution, 1-step	8.1	27.4	9710	10,300
C-26	15	Solution, 1-step	7.3	25.3	9180	10,400
C-27	10	Suspension	5.1	24.4	9860	8,900
C-16	10	None	8.1	27.9	8260[c]	11,200
C-35	10	None	6.0	27.0	5480[c]	7,890

[a] For solution-hardened material, the concentration of formaldehyde was 4%; for suspension-hardened material it was 10 percent.
[b] Control: The casein was dissolved in alkali and treated in exactly the same manner as in the procedure with potassium cyanate except that none of this reagent was added.
[c] These two values are from two separate sets of moldings, and each value is the average of 12 measurements. The individual values are erratic and undoubtedly low because of the tendency of the specimen to develop cracks during its preparation and conditioning.

water content in our specimens necessitated close scrutiny of the conditioning procedure employed. Comparison of tensile strengths could easily be vitiated by different water contents. Generally, moldings were done with powders containing 20% water, whereas the molded specimens equilibrated with an atmosphere of 50% relative humidity and 25° C. would have a content of only about 10% water. The problem was to determine how much time specimens would require to come into equilibrium under these standard conditions of moisture and temperature. Table II shows that there was no appreciable difference in the strength of our spindle specimens conditioned for four weeks or one week. A conditioning period of one week was therefore established as a safe minimum time to obtain equilibrium, at least in the crucial central part of the test spindle. In practice, the test specimens were conditioned according to the A.S.T.M.[13] method for type R materials for at least a week and were then tested under the same conditions, namely, 50 ±2% relative humidity at 25 ±1° C. The tensile strength figures in the tables are averages of at least five specimens.

Flexural strength (modulus of rupture) was determined under the same conditions of temperature and humidity described above. The A.S.T.M. method[11] as designed for 0.5 by 0.5 by 5-in. molded bars was followed, except that the conditioning period was extended to two weeks and that the number of tests averaged was limited to three. The testing machine used was that described by Brother, Suttle and McKinney[14]. The flexural strengths (Table IV) are of interest for comparative purposes, but since they are in most cases about equal to the corresponding tensile strengths they are doubtless somewhat low in an absolute sense. This is probably due to the difficulty of properly conditioning the test bars. Although the conditioning period of a week was shown to be sufficiently long to permit equilibration of the moisture in the tensile test specimens with that of the conditioning atmosphere, it was recognized that such equilibration could not be attained with the thicker flexural test bars even in two weeks; nor was equilibration to be expected in any reasonably longer time. For convenience, therefore, the conditioning period was kept uniformly at two weeks.

Molds for all test specimens met A.S.T.M.[15] specifications. The procedure used for molding all the test specimens, as previously described[16], involved a maximum pressure of 5000 p.s.i., a temperature of 117 to 120° C. and, except as otherwise stated, a water content of 20 percent. It was not always necessary to use 20% water at molding to obtain good flow, but this condition was adhered to so that all results would be on a comparable basis. The percent moisture was determined by drying to constant weight at 105° C.

Properties of molded casein plastics

In preparing protein molding powders, Brother and co-workers[6, 17] generally hardened the soybean protein or casein by immersing the air-dried material in two parts, by weight, of 40% formaldehyde at room temperature for 24 hours. These authors reported no figures on strength of molded specimens. We found the tensile strength of such specimens made from casein previously treated with formalin as described by them to be about 5000 p.s.i. Consequently, it was first thought that the increase in tensile values to 9000 to 10,000 p.s.i. (Exp. Nos. C-10, C-42, etc. of Table IV) obtained for molded samples from casein which had been treated with both potassium cyanate and formaldehyde were due to the cyanate treatment. Control experiments (Exp. Nos. C-30, C-45 of Table IV), in which casein was subjected to the same conditions of pH, temperature, etc., but without potassium cyanate, showed, however, that some factor involved in the hardening procedure had a profound effect on the strength of this material.

Table III shows the effect of the physical condition of the casein during formaldehyde hardening on tensile strength. Commercially dried casein hardened with 10% formaldehyde (Exp. No. C-32) gave specimens having a tensile strength of 7220 p.s.i. If the casein were dissolved and precipitated (Exp. No. C-31) or hydrated by "swelling" in water for 3 hr. (Exp. No. C-74), a noteworthy improvement in tensile strength resulted. Another sample (Exp. No. C-73) was dissolved, precipitated, and dried under mild conditions. It had significantly lower tensile strength than the comparison sample (Exp. No. C-31), which was treated identically except for the drying step. It is apparent from these data that the physical condition of the casein during hardening has an appreciable effect on the tensile strength of the molded product. If due consideration is paid to this effect, tensile strengths in the range of 8000 to 10,000 p.s.i. can readily be obtained. Regarding hardening of casein in solution with formaldehyde, a point which is not dealt with particularly in Table IV should be mentioned, namely, that the pH of the solution (subsequently adjusted to the isoelectric pH) apparently has little effect on strength or other properties of the molded product.

Preparation of carbamido casein

Isocyanate reagents, especially phenyl isocyanate, have been frequently employed to incorporate new groups in proteins. Generally they have been used

13"Tentative methods of conditioning and classifying for conditioning plastics and electrical insulating materials for testing," A.S.T.M. Designation: D 618-44T.
14"A simple flexure-testing machine for plastics," by G. H. Brother, W. C. Suttle and L. L. McKinney, A.S.T.M. Bull. No. 109, 13-16 (1941).
15"Tentative specifications for molds for test specimens of molding materials used for electrical insulation," A.S.T.M. Designation D 647-42T.
16"Water absorption of plastics molded from acylated casein," by W. G. Gordon, A. E. Brown, and C. M. McGrory, Ind. Eng. Chem. 38, 90-94 (1946).
17"Casein plastics," by G. H. Brother, Ind. Eng. Chem. 32, 31-33 (1940).

in an aqueous medium. Thus, Hopkins and Wormall[18] treated casein and other proteins with phenyl isocyanate and p-bromophenyl isocyanate at pH 8 and 0° C. The product of reaction with the latter reagent contained bromine approximately equivalent to the decrease in free amino group. The same authors[19] later obtained immunological evidence emphasizing the ε—amino group of the lysine unit in proteins as the site of reaction. Similar results pointing to the role of the amino group have been reported in the reaction of isocyanates, mostly the phenyl derivative, in aqueous media with insulin[20], horse serum albumin[21], tobacco mosaic virus[22], and egg albumin[23]. Fraenkel-Conrat, Cooper and Olcott[23] have found that the —SH groups of protein may be substituted under certain conditions in water solution and that still other groups may react when the solvent is anhydrous pyridine. Search of the literature, however, revealed no reference to the introduction of unsubstituted carbamido group into protein by reaction of its free amino group with an appropriate reagent. It was necessary to devise a procedure based on those used for amino acids.

Carbamido casein was made by treating acid or rennet casein with potassium cyanate. In the work reported here acid casein was used, since rennet casein gave a product with inferior properties. One kg. of a high-grade commercial acid casein was stirred mechanically into 10 l. of water containing 100 g. of potassium cyanate. First it was stirred at room temperature for 15 min. to allow the casein to swell. Steam was then applied to the jacketed vessel and, with continued stirring, the temperature was raised to 85° C. in the course of 40 minutes. Heating was continued at this temperature long enough to make a total heating period of 2 hr., during which time the casein dissolved. At this point, the pH of the solution was 7.2 to 7.5. The modified casein was then diluted with an equal volume of water, causing the temperature to drop to about 45° C., and 3 N hydrochloric acid was slowly added with vigorous stirring to precipitate the product at pH 3.3 to 3.8. The precipitate was then filtered, washed with water until free of chloride ion, sucked "dry" and dried at not more than 40° C. in an oven with mechanical circulation of air. The weight of product was slightly greater than that of the casein used on a moisture-free basis. Analyses of two preparations of carbamido casein showed, respectively, 2.08 and 2.10% ash (moisture-free basis, as determined by calcium acetate procedure) and 15.46 and 15.29% nitrogen (moisture and ash-free basis),

whereas the figures for the original casein were 2.43 and 15.09. Other preparations were made in exactly the same fashion as described above except that 50 or 150 g. of potassium cyanate were used per kilogram of casein.

Preparations were also made of carbamido casein treated with formaldehyde. Just previous to the final washing, as described above, the carbamido casein was suspended in sufficient water to give a total volume of 13.5 liters. It was then dissolved by the dropwise addition of 2 N sodium hydroxide until the solution was just faintly basic to phenolphthalein. To this solution was added 1.5 l. of 40% formalin, giving a 4% formaldehyde solution. The mixture was held at 60° C. for a total heating time of 2 hr.; then precipitated, filtered, washed, and dried in the manner previously described.

This procedure has been designated as the "two-step" solution method. The "one-step" solution method is identical with the above except that the carbamido casein is not precipitated and redissolved before hardening in solution with 4% formaldehyde. The two methods differ in the following respects: in the former, formaldehyde hardening is done at an initial pH of 8.5—9.0; in the latter, the hardening is done at an initial pH of 7.2—7.5 and, in addition, in the presence of small amounts of by-product urea and ammonia.

Formaldehyde hardening was also done on the material in suspension. In this case, the moist cake of twice-washed carbamido casein was made into a 10% slurry in a 10% solution of formaldehyde, and let stand for 3 days at room temperature. The product was filtered, washed and dried as usual.

Evidence of substitution with carbamido group

Determination of the nitrogen content has often proved useful in following the chemical modification of proteins. However, it was found that the change in nitrogen *percentage* entailed by the conversion of casein to carbamido casein was hardly sufficient for convenient estimation of the amount of substitution. Consequently, attention was turned to the method of Doherty and Ogg[24] for determination of amino nitrogen in insoluble proteins. Figure 2 shows results obtained from two samples of carbamido casein, (prepared in the same manner) indicating roughly that about 80% of the free amino group has been substituted. The curves, which differ from that for casein itself, were compared with those for two model substances, hydantoic acid and ε-carbamido-n-caproic acid. These compounds were prepared by the treatment of glycine and ε-amino-n-caproic acid, respectively, with potassium cyanate. The melting points of 180 and 179 to 181° C., respectively, agreed with those recorded in the literature, and the values for nitrogen of 23.95 and 15.93% agreed with the theoretical values. Both these substances react to a

[18]"Phenyl isocyanate protein compounds and their immunological properties," by S. J. Hopkins and A. Wormall, Biochem. J. 27. 740-753 (1933).
[19]"Phenyl isocyanate protein derivatives and their immunological properties. III. The amino-acid derivatives and serological inhibition tests," by S. J. Hopkins and A. Wormall, Biochem. J. 28. 228-236 (1934).
[20]"Studies on crystalline insulin. XVIII. The nature of the free amino groups in insulin and the isolation of phenylalanine and proline from crystalline insulin," by H. Jensen and E. A. Evans, Jr., Biol. Chem. 108. 1-9 (1935).
[21]"The conjugation of horse serum albumin with 1,2-benzanthryl isocyanates," by H. J. Creech and R. N. Jones, J. Am. Chem. Soc. 62. 1970-1975 (1940).
[22]"Derivatives of tobacco mosaic virus. I. Acetyl and phenylureido virus," by G. L. Miller and W. M. Stanley, J. Biol. Chem. 141. 905-920 (1941).
[23]"Action of aromatic isocyanates on proteins," by H. Fraenkel-Conrat, M. Cooper, and H. S. Olcott, J. Am. Chem. Soc. 67. 314-319 (1945).

[24]"Modified amino nitrogen apparatus for insoluble proteins," by D. G. Doherty and C. L. Ogg, Ind. Eng. Chem., Anal. Ed. 15. 751-753 (1943).

considerable extent with nitrous acid (Fig. 2). Presumably the carbamido group in protein would do likewise, so that the figure for free amino group would have to be revised downward and that for carbamido group upward. The close similarity of the graphs for carbamido casein and for ε-carbamido-n-caproic acid suggests that the ε-amino group of the lysine unit of protein has been largely converted to the carbamido group. The virtual cessation of nitrogen liberation from hydantoic acid at about two-thirds of an equivalent may possibly have its explanation in partial cyclization to the less reactive hydantoin. The graphs for formaldehyde-hardened carbamido casein and formaldehyde-hardened casein of Fig. 2 speak for the reaction of formaldehyde with the carbamido as well as the amino group.

The altered base-combining capacity of carbamido casein is shown by the difference in the titration curves of carbamido casein (Preparation No. C-35) and unmodified casein of Fig. 3. The original pH was 5.30 for the unmodified casein and 5.35 for the carbamido casein, and complete solution was not attained until the pH reached about 6.5. The value of 4.5 ml. of 0.1 N NaOH per gram represents the difference of the two substances between pH 7.2 and 9.5; this difference is equivalent to 0.63% of amino nitrogen, which may be regarded as replaced with the non-dissociated urea group. In other words, these data indicate that at least two-thirds of the amino groups are converted to carbamido radicals. An analysis of other titration curves in which the starting pH of each solution was 6.75 gave results in agreement with the above. A similar result was obtained by formol titration. The diacetyl monoxime colorimetric method for carbamido group[25, 26] was also applied, a color filter and photoelectric colorimeter being used. The values obtained were hardly quantitative but gave qualitative confirmation of extensive incorporation of the carbamido group.

Properties of molded carbamido casein

Certain physical properties of plastics molded from several different preparations of carbamido casein, as compared with those of casein, are listed in Table IV. The results indicate that treatment with 10 g. potassium cyanate per 100 casein is somewhat more effective than with 5 g. of the reagent in curtailing water absorption. Hardening with formaldehyde, which accomplished considerable reduction in water absorption of unmodified casein and even of acetylated casein[16], has little similar effect on higher fatty acid derivatives[16, 27] or on carbamido casein. This lack of effect on the last-named substances is consistent with elimination of the large water-absorbing tendency of the amino group[28]. On the other hand, analysis of the formaldehyde-hardened carbamido casein for formaldehyde showed that the formaldehyde-binding capacity of casein (compare footnote 29) is not much altered in the conversion to carbamido casein. This is in contrast to the effect of acylation[16, 30] in decreasing the affinity for formalde-
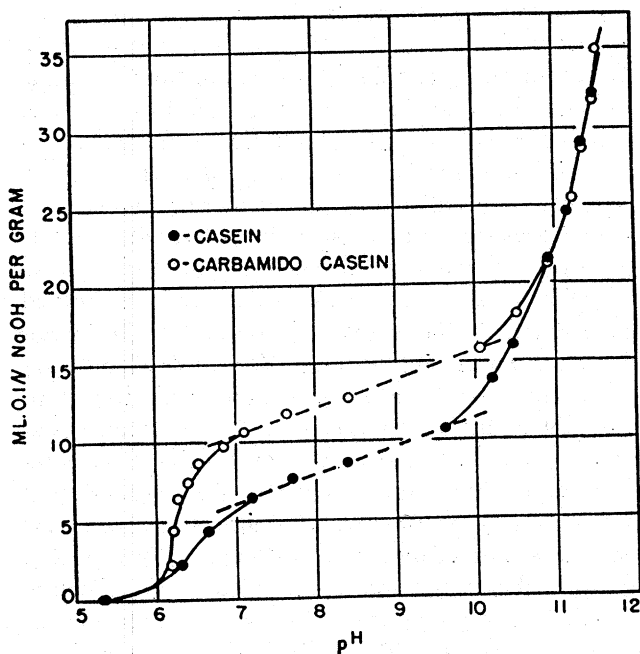
3 — Titration curves for casein and carbamido casein

hyde. The evidence here, to be sure, is rather indirect but does point to the reaction of formaldehyde with the carbamido group in the protein. Hardening of a suspension of carbamido casein with formaldehyde results in somewhat lower water absorption than hardening in solution; strength values are about the same in both cases. The strength of carbamido casein which has not been hardened with formaldehyde is definitely lower than that of the hardened material. The individual values on the former are erratic and undoubtedly low because of the tendency of the material to check during preparation and conditioning of the specimen. The main value of the formaldehyde hardening of carbamido casein is to increase durability and toughness.

Value of plasticizers

The equilibrium water content of many protein plastics, including those described in this paper, is about 9 to 10% at 25° C. and 50% relative humidity. As a consequence, moldings made with a 20% water content lose water and shrink under standard testing conditions as well as under ordinary atmospheric conditions. Shrinkage may be accompanied by warping. Dimensional stability can be obtained by limiting the water content to 10% and replacing the water required above this level with a non-volatile

[25]"The carbamido diacetyl reaction: A test for citrulline," by W. R. Fearon. Biochem. J. 33, 902-907 (1939).
[26]"A colorimetric method for the determination of citrulline," by A. G. Gornall and A. Hunter. Biochem. J. 35, 650-658 (1941).
[27]"Plastic properties of higher fatty acid derivatives of proteins," by W. G. Gordon, A. F. Brown, C. M. McGrory, and E. C. Gall. Ind. Eng. Chem. 38, 1243-1245 (1946).
[28]"Water absorption of proteins. I. The effect of free amino groups in casein." by E. F. Mellon, A. H. Korn, and S. R. Hoover. J. Am. Chem. Soc. 69, 827-831 (1947).
[29]"Combination of formaldehyde with casein," by A. P. Swain, E. L. Kokes, N. J. Hipp, J. L. Wood, and R. W. Jackson. Ind. Eng. Chem. (in press).
[30]"Higher fatty acid derivatives of proteins," by W. G. Gordon, A. E. Brown, and R. W. Jackson, Ind. Eng. Chem. 38, 1239-1242 (1946).

organic plasticizer. Without organic plasticizer, a water content approaching 20% is required, particularly with formaldehyde-hardened casein, to obtain good plastic flow, as shown by the molding characteristics of disks molded 1/16 in. thick for the water-uptake tests. The dimensional stability of moldings made with 10% water in contrast to those made with 20% water was determined by measurement of the diameter of the tensile strength specimens (spindles) before and after conditioning at 50% relative humidity and 25° C. Our conditioned spindle specimens molded with 10% water did not change in diameter, whereas those molded with 20% water decreased in diameter 0.006 in., or 3% of the total.

Many of the substances so frequently referred to in the literature[1, 31] as plasticizers for casein, including polyalcohols and a variety of substituted amines, invariably increase the water uptake of the molded casein plastic. Among the compounds which do not affect the water uptake adversely and which may be incorporated in a simple manner is the commercially available Santicizer-9 [32] (hereinafter referred to as S-9), a mixture of o- and p-toluenesulfonamide. Preliminary experiments showed that organic plasticizers cannot be adequately mixed with casein in a mortar or ball mill. In the work reported here the plasticizer was introduced in an effective manner by co-precipitating the casein and plasticizer. Santicizer-9 and related plasticizers are particularly amenable to this method because of their solubility in alkali. When the protein material was hardened in solution with formaldehyde, S-9 was added after the formaldehyde but before precipitation; when hardening was done with the material in suspension, the S-9 was added before precipitation with acid and treatment with formaldehyde.

In contrast to the results obtained with S-9 and commercial soybean protein by others[33], our experiments showed that S-9 materially improved the water uptake and the flow of casein and carbamido casein. The decrease in water uptake (Table V) was greater than would be expected if the S-9 acted merely as an inert filler. The improvement in the values for the 24-hr. water absorption, as compared with the values for the approximate saturation water content, shows that the rate of water uptake was substantially affected by the plasticizer, S-9. The tensile strength was not decreased in moldings having a plasticizer content as high as 8% and, as other experiments have shown, suffered only a small loss when the plasticizer content was raised to 20 per cent. The reduction in water uptake for equal amounts of S-9 was greater with formaldehyde-hardened carbamido casein than with formaldehyde-hardened casein. As pointed out above, this plasti-

cizer was a mixture of o- and p-toluenesulfonamide. When it was substituted with one ethyl radical in the amide group, the product appeared a little less satisfactory. The dialkyl substituted amide did not even seem compatible with casein and its derivatives.

The economics of plasticizing casein and carbamido casein with S-9 by co-precipitation are not favorable to large-scale application. However, commercial casein or carbamido casein like the cellulose and vinyl resins, can be plasticized on heated differential rolls. The hardening agent, paraformaldehyde, may be added to the roll-milling mixture or the required amount of formalin may be added to the plasticized material after roll-milling to complete the preparation of the plasticized casein molding powder. Detailed reporting of the particulars concerning this phase of the investigation and the large-scale preparation of carbamido casein will be deferred.

Practical effect of modifying treatments

The data show that the affinity of casein for water is substantially reduced by introduction of just the carbamido group. This reduction in water uptake is greater than that which can be accomplished by treatment of casein with formaldehyde alone. Moreover, the adhesive properties of carbamido casein are not destroyed, whereas formaldehyde-treated

Table V.—Effect of Added Plasticizer on Physical Properties of Carbamido Casein and Casein Plastics[a]

Exp. No.	Formaldehyde hardening of protein	Plasticizer in plastic[b]	A.S.T.M. 24-hr. water absorption	Approx. satn. water content	Tensile strength	Reduction in approx. satn. water content[c]
		%	%	%	p.s.i	%
			Casein			
C-30	Solution	0.00	15.3	33.8	9,500	
C-38	"	1.95	13.4	30.5	8,900	10
C-38-1	"	4.68	11.5	29.2	8,500	14
C-46	"	5.07	10.7	28.2	9,550	17
C-31	Suspension	0.0	11.4	30.9	9,550	
			Carbamido Casein[d]			
C-29	Solution	0.00	7.9	26.7	9,500	21
C-29P	"	0.56	7.5	27.0	9,500	20
C-34a	"	2.06	6.5	23.5	9,850	30
C-34b	"	3.79	5.8	22.7	10,300	33
C-34c	"	8.42	4.7	21.0	9,500	38
C-27	Suspension	0.00	5.1	24.4	9,860	21
C-40	"	1.39	3.5	20.9	....	32

[a] Plasticizer added prior to precipitation, and co-precipitated with product. Although the co-precipitation method yields homogeneously plasticized products, the method is not economical for S-9, notwithstanding the rather low solubility of S-9 in water. The specimens without S-9 (also specimens C-29P) were molded with 20% water, and those containing S-9 with 10% water.
[b] Calculated from sulfur analysis.
[c] In calculating reduction of water uptake, all preparations hardened with formaldehyde in solution are compared with C-30; all hardened in suspension are compared with C-31.
[d] One-step procedure, 10 g. KCNO per 100 casein.

31."La plastification de la caseine," by J. Delorme, Rev. Gén. Mat. Plastiques 11, 375-377, 379 (1935).
32The mention of commercial products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over others of similar nature not mentioned.
33"Protein plastics from soybean products. Plasticization of hardened protein material," by G. H. Brother and L. L. McKinney, Ind. Eng. Chem. 31, 84-87 (1939).

casein does not exhibit any appreciable adhesion. The utility of carbamido casein, with its reduced affinity for water, for applications in which adhesive qualities are essential, has not been fully evaluated. However, carbamido casein has been extruded in the same manner as casin to produce monofils or bristles, and unlike acetyl or benzoyl casein, the textile fiber from carbamido casein is not weak and brittle.

Moldings made from carbamido casein not treated with formaldehyde check and become brittle, but moldings made from the formaldehyde-treated material are tough and durable, although the water uptake is essentially unchanged. As with casein, formaldehyde treatment affects the plastic flow of carbamido casein but to a less degree. A good estimate of the relative plastic flow can be obtained by observing the molding of thin discs 2 in. in diameter and 1/16 in. thick). Although the plastic flow of formaldehyde-carbamido casein is superior to that of formaldehyde-casein, moldings of the former may require more than 10% water; if so, they will shrink when conditioned[13] or when subjected to ordinary atmospheric conditions. The carbamido substituent materially improves molded specimens saturated with water. Water-absorption specimens of unhardened or formaldehyde-hardened carbamido casein when allowed to stand in water at 25° C. until saturated are rigid and increase 0.03 in. per in. in diameter, as compared with formaldehyde-hardened casein specimens which are rubbery and increase 0.12 in. per in. diameter. The latter specimens were attacked by micro-organisms, but the former specimens did not show any evidence of micro-organism attack even after being in the same water-saturated atmosphere for three years.

Conclusions

Direct moldings of simple objects can be made from casein and carbamido casein which have been prehardened with formaldehyde. The modification of casein by incorporation of the carbamido residue yields molded materials superior to formaldehyde-hardened casein in respect to water absorption without affecting strength. Moldings of materials prehardened with formaldehyde containing 10% water in conjunction with an organic plasticizer are dimensionally stable, and the water uptake of these materials, particularly carbamido casein, is substantially reduced by the organic plasticizer. Athough treatment of carbamido casein with formaldehyde does not cause an additional decrease in water absorption, it results in a substantial improvement in toughness and durability. In this connection data are presented indicating that the ureido group reacts with formaldehyde. Carbamido casein possesses the properties of casein which make it valuable as a raw material for many industrial applications. In some respects, carbamido casein is superior to casein.

Acknowledgment